# SPM2

# User guide for fMRI

Cyril Pernet
INSERM Unit 455, Université Paul Sabatier, Toulouse, France
Brain research Unit, Low Temperature Laboratory, Helsinki University of Technology, Espoo, Finland
cyril@neuro.hut.fi

# Index

**I. Preparing images before preprocessing**
1.1. Artefacts in images
1.2. Orientation and origin of images

**II. Preprocessing**
2.1. Slice timing correction: correction of the acquisition time delay between slices of a volume
2.2. Realign: movement correction
2.3. Coregister: alignment of functional and anatomical data
2.4. Normalize: all brains in the same space
2.5. Smoothing

**III. Rendering of 3D high resolution images**
3.1. Segmentation
3.2. Rendering

**IV. Individual Statistical Analysis**
4.1. Model specification
4.2. Contrasts

**V. Random effect Analysis**
5.1. Statistical tests
5.2. 'Basic' example

**VI. Statistical Issues**
6.1. Use of derivatives
6.2. Correction for multiple comparisons
6.3. Sphericity correction
6.4. Conjunctions

**VII. Results and Graphs**
7.1. Examine results
7.2. Display results

I wish to acknowledge the contribution of **Simo Vanni** (AMI center, Low Temperature Laboratory, Helsinki University of Technology, Espoo, Finland) for his help to improve the manuscript and **Thomas Nichols** (Department of Biostatistics University of Michigan, MI, USA) for his kind advices and corrections on statistical issues.

This work is inspired from the French SPM99 manual hosted by the Centre d'IRM Fonctionnelle Cérébrale, Marseille, France.
(http://irmfmrs.free.fr/formation/traitement_des_donnees/spm99doc/)

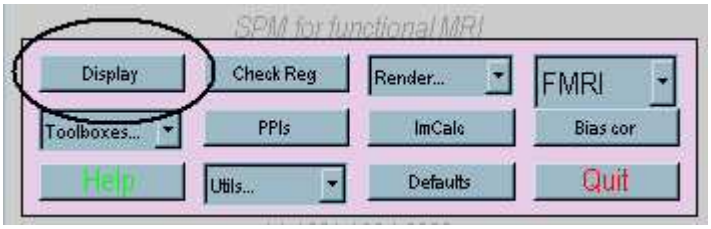
# I. Preparing images before preprocessing

## 1.1. Artefacts in images

Sometimes acquired images have inadequate quality for technical reasons. Before working (for hours), it could be useful to check images from each session … (one image by session is generally enough as problems do not appear on one image but generally on time series). During fMRI acquisition, initial images of each session show some artefacts related to signal stabilization. These images have to be deleted! Remove at least images acquired during the first 6 seconds of acquisition (i.e. the three initial volumes if the interscan interval (TR) is longer than 2 seconds or more for TR < 2 seconds)

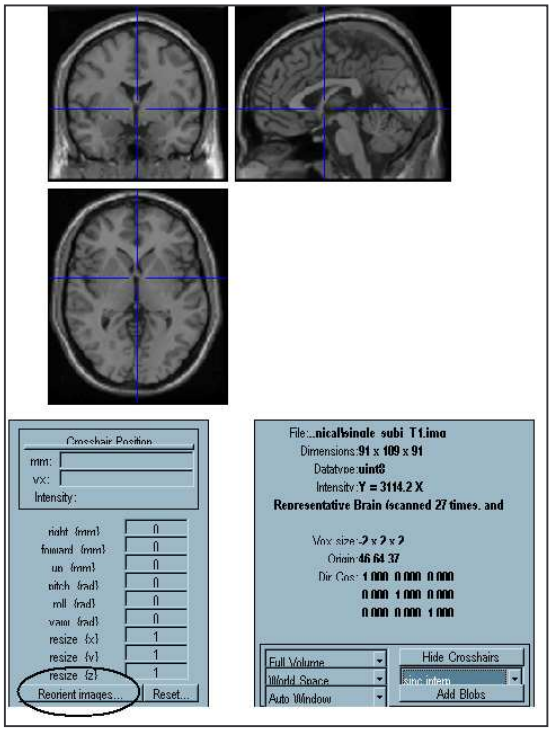
## 1.2. Orientation and origin of images

All images coming from one subject and from all subjects have to be oriented in the same direction. To look at your images, choose the **DISPLAY** button. After selecting an image, SPM will show the image in the transverse, sagittal and coronal views. Images have to be oriented with the left side of the brain in the ride side of the image, the nose on the left side and the top of the head on the top of the image.




When images are in neuroradiological convention (this is the default output of the scanners), the defaults.analyze.flip should be set to 1 (by default it's 0).

In the graphical window, two different control panels are available. Within the ride side panel, the file name, the matrix dimension, the data type, the intensity, the voxel size and the origin (in voxel) of the displayed image are presented. When images are correctly oriented, the matrix direction should show on the diagonal 1, 1, 1. To reorient images, modifications can be applied onto images (translations or rotations) in the left sided control panel (the ride sided menu is used to look at brain activation) and then click on **REORIENT IMAGES** and choose images to reorient. Be careful, a .mat file is created to code the orientation. SPM will show the data in a different orientation but they are still coded in the header in their initial orientation (as you can check with MRIcro).




It is also important to have the same origin for all images. This point (0, 0, 0 in millimeters and defined in voxels in Analyze format) is used by SPM to locate each fMRI images onto the anatomical one. This point is defined in the image.hdr file. Choose **set origin** … and apply to all images for a subject (headers of all files are then update with the new origin).

Check transformations using the **CHECK REG** button. Choose anatomical and functional images. Then move the crosshair on images and verify that images are similar in dimensions and coregistered properly.

# II. Preprocessing

There is no conventional preprocessing procedure. The order and the choice of preprocessing steps depend on what you are interested in. However, according to 'classical' analyses of fMRI data, it is possible to define a 'standard' procedure.

If the data are acquired in an *interleaved mode* then first begin by the slice timing correction (2.1), second perform the realignment (2.2), next the coregistration of fMRI data with anatomical ones (2.3 - optional), the normalization (2.4) and finally the smoothing (2.5). If the data are acquired in a *sequential mode* then first begin by the realignment procedure (2.2), second the coregistration (2.3 - optional) and then the slice timing correction (2.1). Next, fMRI data have to be normalized (2.4) and smoothed (2.5).


## 2.1. Slice timing correction: correction of the acquisition time delay between slices of a volume

Choose **SLICE TIMING**

**Number of subjects / sessions:** select time series to correct

**Acquisition order:** write the sequence of slice acquisition of your magnet (e.g.: 1, 3, 5, 7, 9, 11, 13, 15, 2, 4, 6, 8, 10, 12, 14, 16 for interleaved 'mosaic' sequence on Siemens magnet)

**Reference slice:** this corresponds to the slice used for estimating time correction – choose the slice acquired in the middle of the time sequence (in the previous example, the number 15)

**Interscan interval (TR) (secs):** this corresponds to the time between the acquisition of two volumes

**Acquisition time (TA) (secs):** this corresponds to the time of acquisition of one volume (default is TA = TR – TR/Number of slices)

*New images are then created (aimage.img and aimage.hdr).*

*This correction allows obtaining identical time points for all slices of a given volume. This correction has to be done after the realignment and coregister procedures if data are acquired in a sequential mode because, without movement correction, the slice timing would perform a temporal interpolation on data coming from spatially different voxels. Conversely for fMRI data acquired in an interleaved mode, the slice timing have to be done before realignment and coregister procedure because the movement correction procedure (if done before slice timing) could move temporally different voxels (max TR/2) from one slice to another.*

## 2.2. Realign: movement correction

Choose **REALIGN**

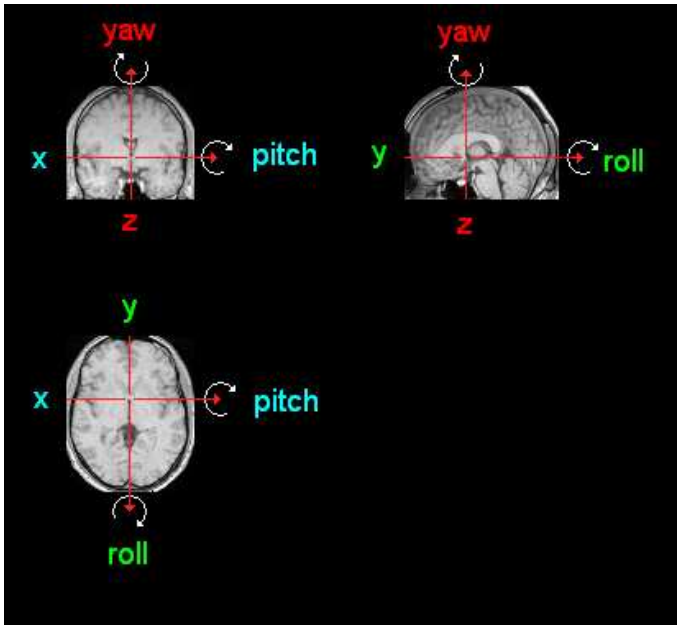
**Number of subject:** X

**Number of sessions for subject 1:** choose the number of experimental sessions to correct and select SCANS FOR SUBJ1, SESS1. For each subject, the first selected session will be the reference session (choose the session acquired just before/after the anatomical session). For each session, there is also a reference volume. Click on SUMMARY VEW → CLICK TO EXPAND → choose the reference volume (the central volume in the time series) → ALL.

**Which option:** COREGISTER ONLY (only compute the transformation matrix) or COREGISTER & RESLICE (compute and propose 'create what')

**Create what:** ALL IMAGES + MEAN IMAGE (for sequential mode of acquisition or interleaved mode if no normalization is required) or MEAN IMAGEs ONLY (for interleaved mode).

*New files are written, either raimage [.img, .hdr] and the mean_image [.img, .hdr] or rimage.mat and the mean_image [.img, .hdr]. When the reslice step is not applied (COREGISTER ONLY), SPM computes the transformation matrix for each image only (this is the rimage.mat files). The realignment procedure is based on translations and rotations in X, Y, Z. An SPM.ps file is created where translations (X blue, Y green, Z red) and rotations (X blue = pitch, Y green = roll, Z red = yaw) are given for each session.*



*The realignment procedure is based on a similarity measurement between images. SPM used an iconic approach (or intensity based approach) where some voxels are chosen in a source image and 'modified' according to the intensity of a reference image. It is also sometimes possible that the movement estimation is biased by activations. An SPM compatible toolbox (INRIAlign) was developed to minimize this problem. In SPM, as in the INRIAlign toolbox, each functional image is aligned, within each session, onto the reference volume. Similarly, each functional image is aligned, across sessions, onto the reference session. The transformation matrices will be applied by an interpolation method to create new image. When working with data acquired in an interleaved mode, the best solution is to compute the realignment parameters, next the coregistration and the normalization parameters. Only one resampling is then applied.*

With the **DEFAULTS** button, realignment options are available

**Registration Quality?** From 0,001 to 1

**Allow weighting of reference image?** Voxels of the reference volume will be weighted according to the estimated variance between volumes of each session … could be useful when lot of movement are observed

**Reslice interpolation method?**

- Nearest Neighbour,
- Trilinear Interpolation (leads to a smoothing on some voxels .. could be useful for PET)
- B-spline (degrees 1 to 7), the best interpolation method (for me) except for artefacted images … (the use of too high polynomial function (i.e. degree 7) is not a good option as it will create too many new points with higher or smaller intensities between original points).
- Fourier space Interpolation (only for isotropic voxels)

**Way to wrap image?** Correction of fMRI inhomogeneities

**Mask images?** The mask is used to select only voxels common to all images of a subject

## 2.3. Coregister: alignment of functional and anatomical data

*The coregistration is an optional step. Once T2* images are realigned, one can normalize them onto the EPI template. Another option is to warp the 3D of your subject so that it looks like the mean EPI created during the realignment and then use this warpped 3D to compute the normalization to the T1 template. However, be aware that the coregistration between the T1 3D and the mean EPI does not take well into account the deformation of EPI images.*

Choose **COREGISTER**

**Number of subject:** X

**Target image:** select the reference EPI (most of the time the mean EPI image) of the subject

**Source image:** select the T1 MRI image of the subject

**Other images:** by default press done without selecting any other images or if you need, you can select other images to transform using the same matrix as for the T1 MRI image

*This step is used to coregister images from the same modality or from separate modalities. For fMRI, functional images (T2*) are coregistered with anatomical images (T1). As image intensities between T1 and T2 are not the same, it is not possible to use a 'simple' intensity based procedure as for realignment. SPM2 uses mutual information as similarity measurement*

## 2.4. Normalize: all brains in the same space

### Choose the default parameters

**DEFAULTS** → default area? → spatial normalization → default for?

**WRITING NORMALIZED**

**Preserve what?** Concentrations

**Bounding Box?** This determines the size of the normalized volume. When the anatomical image (T1 3D) is acquired during the same exam as functional images, the TEMPLATE (-90:91 -126:90 -72:108) model can be used. If it is not possible to use a 3D, choose the EPI template.

**Voxel Sizes:** this option allows you to choose the size of the voxels.

**Interpolation Method?** Here default is trilinear (personally, I use 4$^{th}$ degree B-Spline which tends to give better results)

**Way to wrap images?** No Wrap

### Normalize and Write the data

Choose **NORMALIZE**

**Which option?** Determine parameters and write normalized

**Template image** (choose T1 or EPI)

**Source image** (choose the r3D of your subject)

**Images to write** (choose EPI images of your subject)

*SPM computes a transformation matrix between the r3D image and the T1 template from the MNI and applies transformations onto EPI images. If the realignment was computed and not applied, then SPM will create new realigned and normalized images (waimage). All transformation matrices (realignment matrix and normalization one) will be multiplied and only one transformation will be applied onto data. As transformations do not concern full voxels, this procedure leads to less interpolation errors than a "compute and apply" systematic routine.*

*Note that if your data are in the neuroradiological convention, after the normalization procedure, SPM will show the data in the neurological convention. The spatial normalization procedure puts all images in the same space (the MNI space). Data stored as right- or left-handed are both interpreted in a right-handed coordinate system through a "voxel-to-world transformation". All the computations are performed on coordinates within the right-handed system. During this procedure, SPM creates a mask (mask.img) that can be read with the DISPLAY option when statistical analyses are done. Indeed, statistical analyses will be computed for voxels within this mask only. This mask corresponds to all intersected normalized volumes. As the normalization is computed with the MNI space as reference and as this template use the AC/PC plane as reference, it is better to acquire your data along this plane. Strong variations along the AC/PC plane during the data acquisition could put some slices outside the mask for statistical analyses.*

## 2.5. Smoothing

Choose **SMOOTH** and enter the size of the Gaussian spatial filter (FWHM: full width at half maximum).

For a single subject study or for very focal activations in a group study, FWHM could be 1.5x the size of the normalized voxel. For more classical group analyses, FWHM could be 3x the size of the normalized voxel.

*This step allows processing the spatial correlation across voxels using the random field theory during the thresholding of 'activation maps'.*

# III. Rendering of 3D high resolution images

## 3.1. Segment

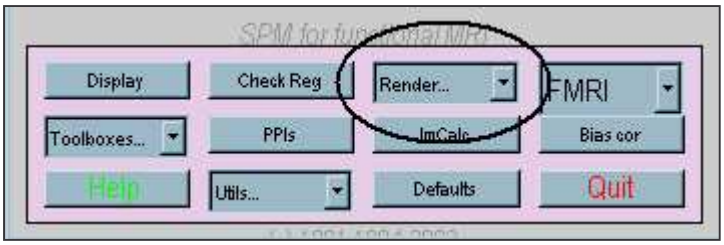
Choose **SEGMENT**

**SELECT MRI(s) FOR SUBJECT 1**: select a T1 image (usually high-resolution 3D)

**ALREADY SPATIALLY NORMALISED?** Yes/No
→ No: **MODALITY?** Choose the modality of your image (here MRI T1)

*SPM will create 3 images: grey matter, white matter and CSF. SPM uses a mixture model to classify voxels in different classes. When the image is not normalized, SPM needs to compute first the transformation matrix between the image and the template (affine transformations only) to get a priori probabilities on the grey/white matter or CSF membership.*

## 3.2. Rendering




**RENDER**

**XTRACT BRAIN**: select image.seg1.img and image.seg2.img (= grey and white matter)

**SAVE:**
- Rendering
- Extracted surface
- Rendering and surface
- Surface as OBJ format

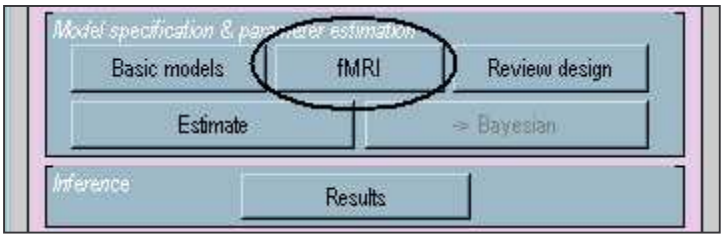
Then you can look at the render using the display option RENDER DISPLAY …

# IV. Individual Statistical Analysis

Don't forget to specify the defaults parameters!

**DEFAULTS → STATISTICS – FMRI →**

**UPPER TAIL F PROB. THRESHOLD (FMRI):** .001 (default value)
**NUMBER OF BINS/TR:** this corresponds to the number of time points that will be used by TR to construct the hrf (usually = number of slices per volume)
**SAMPLED BIN:** this value corresponds to the starting point of the hemodynamic function, this value is computed as ([(Number of bins/TR)*(number of the ref slice-1)]/Number of slices)+1 .. generally it is the number of the ref slice used during the slice timing (in the example I used in the slice timing, N=16 and the ref. slice is 15, the Number of bins/TR=16 and thus the sampled bin is also 16).




## 4.1. Model specification

**fMRI → specify design or data → design**

- **Interscan interval {sec}** write here the TR
- **Scan per session e.g. 64 64 64 64** this corresponds to the number of images (volumes) you have in each session
- **Specify design in scans/secs** select if you want to specify the onset of each stimulus in seconds or according to volumes of acquisition (1st volume = 0)
- **Are sessions replications yes/no** if the same experimental design is used in each session select 'yes' in the other case select 'no'
- **Select basis set** you have the choice between the hemodynamic response alone or with derivatives (the 1st derivative could capture some temporal differences in the response whereas the second derivative is used to look at the spread of the hrf), or Fourier or gamma functions or a finite impulse model
- **Model interaction (Volterra)** No (used to model non linearities related to interaction between trials)

Session 1: trial specification in scans/sec

- **Number of conditions/trials** enter the number of different experimental conditions
- **Name for condition/trial 1?** name
- **Vector of onset – trial 1** enter the onset of each stimulus of the 1st experimental condition (e.g. for scans 0 8 24 36)
- **Duration[s] (events =0)** enter the duration of the stimulation and 0 for event related designs
- **Parametric modulation none/time/other** select none (used to modulate the regressors)

- ….
- **User specified** you can add other regressors than those chosen to model the hemodynamic response (e.g. 6 motion parameters, type 'spm_load' and chose .txt files - Warning: in the session with the mean image, there is an extra-line at the beginning of the txt file that need to be suppressed)

*SPM creates a SPM.mat file that corresponds to the structure of experimental design and the regressors used to model the hemodynamic response.*

**fMRI → specify design or data →** data

**Select SPM.mat** (you have to select the matrix you've just specified)
**Select scans for session 1** … choose the experimental sessions in the same order as specified in the matrix

**Spm_fmri_spm_ui: Global intensity normalisation…**
**Remove Global effects scale/none** Select none*

**Spm_fmri_spm_ui: Temporal autocorrelation options**
**High-pass filter? none/specify specify → cutoff period (secs)** and enter values for each sessions to remove low-frequency variations (2.5 x maximum SOA length)
**Correct for serial correlations? None/AR(1)** Select AR(1) to correct intrinsic temporal correlations.

*The matrix is then displayed in the graphical window ... save your config using SPM-Print button or File → Save as (be careful, the folder of the graphical window in not always the folder used by Matlab)*

**ESTIMATE** select the SPM.mat

* The question of global normalization has been discussed on the SPM list (February 2005). According to this discussion, I would say that for 'classical' fMRI analyses, there is no need to choose the option "scale images". By default (none button), an overall grand mean (between sessions) is computed to scale all images of subject. If you select the scale button, the mean over all voxels, for all volumes of a session is computed. Then all voxels in all volumes of that session are scaled by this mean. Here, at the individual level, the baseline of a subject should be the same for the different sessions and such scaling is not mandatory.

*During the estimation of β parameters, SPM will create different files*

***beta_###.img*** *&* ***beta_###.hdr****: these images correspond to different regressors of your matrix. Values ### for each file correspond to the number of your regressor in the SPM.mat file. These images can be read by the DISPLAY button.*

***mask.img*** *&* ***mask.hdr****: this file* corresponds to the common image of all volumes of your subject. The mask file is used for statistical analysis. It corresponds to a binary image (0/1) used to determine which voxels have to be analyzed.

***ResMS.img*** *&* ***ResMS.hdr****: this image contains, for each voxel, the estimated residual (or error) variance value.*

***RPV.img*** *&* ***RPV.hdr****: estimated resels per voxel image. The number of resels is used to compute the threshold in the multiple comparison statistics.*

## 4.2. Contrasts

F « effects of interest »

*This contrast will look into voxels where the model « X » fits the signal variations Y (with Y=βX+e). It will display regions where there is activation, i.e. where at least one regressor fits the data.*

For a model with n regressors (conditions) you will have:
H0: βA=0 and βB=0 and … βn=0
H1: βA≠0 or βB≠0 or … βn≠0

This could also be written as:
H0: βA. βA + βB. βB + … + βn.βn = 0
H1: βA. βA + βB. βB + … + βn.βn ≠ 0

F contrast, looking at the difference between two conditions

*The F contrast compares the variances of the residual errors*

H0: β (condition A) = β (condition B) or also (β condition A) - (β condition B) =0
H1: β (condition A) ≠ β (condition B) or also (β condition A) - (β condition B) ≠0

*If each condition has several regressors (hrf + derivatives), the F contrast will then look at the difference of variations for each regressor, with*

H0: ($\beta^1$ condition A) - ($\beta^1$ condition B) =0 and ($\beta^2$ condition A) - ($\beta^2$ condition B) =0
H1: ($\beta^1$ condition A) - ($\beta^1$ condition B) ≠0 or ($\beta^2$ condition A) - ($\beta^2$ condition B) ≠0

T contrast

*You can use the T contrast to look at the difference between two conditions (each one with one regressor). Because the t contrast is a one-tailed test, you can inquire the direction of the difference between two conditions (A>B or A<B).*

H0: β (condition A) = β (condition B) or also (β condition A) - (β condition B) =0
H1: β (condition A) > β (condition B) or also (β condition A) - (β condition B) >0

**Results** → select SPM.mat.

In the **SPM CONTRAST MANAGER**, Choose either F-CONTRASTS or T-CONTRASTS

**Mask with other contrast(s) YES/NO**
**Title for comparison**
**P value adjustment to control FWE/FDR/none**
**Threshold (T or p value)** enter a threshold
**& extent threshold {voxels}** enter the minimum cluster size you want

Mask
*If you choose to mask your data, you can choose between inclusive or exclusive masking procedures. The inclusive masking procedure allows looking for activated voxels in a contrast λ1 under consideration among voxels activated by the contrast λ2 you have selected as a mask. The exclusive masking procedure allows looking for activated voxels in a contrast λ1 under consideration that are different to those activated by the contrast λ2 you have selected as a mask.*

P value adjustment
*Because SPM uses a massive univariate approach, i.e. tests for each voxel the probability to reject the null hypothesis, this induces a multiple-comparison problem that requires a correction. The correction is either based on the Gaussian random field theory (FWE) or on the probability to find false positives (FDR); which in turn decreases the power and the sensitivity for large volume. An alternative to the classical null hypothesis testing procedure is provided in SPM2: the use of Posterior Probability Maps, i.e. the use of Bayesian statistics (Bayesian button)*

During the statistical analysis, SPM will create new files:

**ess##.img & ess.hdr** these images correspond to the difference between two regressors X1 and X2 for F contrasts leading to extra-sum-square or ess images

**spmF_##.img & spmF_##.hdr** these images correspond to the F value for each voxel

**con##.img & con##.hrf** these images correspond to the linear combination of the β parameters for T contrasts

**spmT_##.img & spmT_##.hdr** these images correspond to the T value for each voxel

# V. Random effect Analysis

**BASIC MODEL**

*SPM will use con*.img or ess*.img files to compute the statistical significance of each voxel, based on the estimation of the effect computed for each subject.*

## 5.1. Statistical tests

ONE SAMPLE T TEST
Test the null hypothesis that the mean signal value is not different from 0.

TWO SAMPLE T TEST
Test the null hypothesis that the mean signal value of the group 1 is not different to the mean signal value of the group 2.

PAIRED T TEST
Test the null hypothesis that the mean signal value of the condition 1 is not different to the mean signal value of the condition 2 (same group of subjects).

ONE WAY ANOVA
Test the null hypothesis that the mean signal value of 3 groups/conditions or more are not different.

SIMPLE REGRESSION (CORRELATION)
Test the null hypothesis that the variable 'a' in the linear regression equals 0; with $y = ax + b$ (y is the value of the contrast and x the predictive factor)

MULTIPLE REGRESSION
Test the null hypothesis that the variable 'a' in the linear regression equals 0 for each predictor; with $y = a_1x_1 + a_nx_n + b$ (y is the value of the contrast and $x_{1...n}$ the predictive factors)

ANCOVA
Test the null hypothesis that the mean signal value of the group/condition 1 is not different to the value/condition 2 or more, when the effect of a predictive factor x is controlled.

## 5.2 'Basic' example

Let's imagine an experiment with two groups of subjects A & B, each group of subjects have to perform two tasks 1 & 2. In addition, a baseline condition is measured (condition 3). We have a well-designed study with 6 groups of measures A1, A2, A3, B1, B2 & B3.

For each subject, the model is convolved with the hrf and the matrix is: condition 1, 2, 3. Then you can assess for the effect of the conditions 1 and 2. For the condition 1, you can enter a t contrast [1 0 –1] as well as for the condition 2 [0 1 –1] (let's say con1 and con2). You can also compare conditions 1 & 2 using t or F contrasts: F[1 –1] or T[1 –1] & [-1 1] (ess1, con3 and con4)

At the group level, you can look at the difference between conditions 1 and 2 for one group. Here, you can perform either a one-sample t-test on images con3 or con4 or a paired t-test on images con1 versus con2. The result is the same, as you will oppose the same regressors.

If you want to compare groups A & B in the condition 1, you can perform a two sample t-test on images con1.

A full analysis could also be performed with an ANOVA. You can use 4 conditions, groupA1>3, groupA2>3, groupB1>3, groupB2>3, i.e. con1 & con2 images. Then, you can assess the difference between groups [1 1 –1 –1], between condition [1 –1 1 –1] or the interaction [1 –1 –1 1].

A full description of the different analyses with or without additional regressors … etc is provided on the SPM web site.

# VI. Statistical Issues

## 6.1 Use of derivatives

Derivatives would compensate differences in the timing or the dispersion. In the contrast manager, contrasts have to be done for each regressor [1 0 0], [0 1 0] or [0 0 1].

Regressors should be orthogonal, which means that there is no variance that could be explained by "both" regressors. This means that the inclusion/exclusion of the derivative cannot affect the parameter estimate, so that only the denominator of the test-statistic is affected (test-statistic is of the form (estimated experimental effect) / (estimated error variance)).

Canonical and temporal derivative parameter estimates through to a second-level analysis (1xN repeated measures, within subjects ANOVAs): Choose a one-way ANOVA design (no constant) from the Basic Models option, and select N groups. You should also then allow for non sphericity answering "no" and "no" to the "independent?" and "identically distributed?" questions (since different basis functions typically have different scalings, i.e. different covariances). An F-test in the resulting model will test for any effect of your basis functions. For more complex 2x2x...N ANOVA designs, you need to create a contrast of the parameter estimates for each basis function first, and run a new one-way ANOVA on these contrasts for each effect of interest (e.g. main effect/interaction).

## 6.2. Correction for multiple comparisons

Because multiple comparisons are performed, one needs to correct it. There are two methods for accounting for the multiple comparisons: the family wise error correction and the false discovery rate.

### Family Wise error correction

This correction computes a correction for all voxels controlling the chance of *any* false positives. This correction is based on either Bonferroni or random field Gaussian theory, which ever provides more sensitive inferences. A Bonferroni threshold is found simply as the $\alpha$-level (e.g. 0.05) divided by the number of voxels. When there is spatial correlation (smoothness) however, Bonferroni can be very conservative.

SPM (FWE button) relies on the random field theory. Random field theory can provide more sensitive thresholds by accounting for the smoothness of the data (to be precise, the smoothness of the noise; see smoothing). The smoothness is measured in FWHM of the Gaussian kernel, and RESEL count is number of 'virtual' voxels of size $FWHM_x \times FWHM_y \times FWHM_z$ that would fit into the brain. Using the expected Euler characteristic (it corresponds roughly to the number of blobs in a null statistic image after it has been thresholded), a threshold is determined that controls the chance of a family wise error (see the Cambridge imagers website for an excellent introduction).

In short, use of a 0.05 Family wise error threshold ensures, over many replications of an experiment, no more than 1 out of 20 (5%) of the experiments will have any false positives.

**False discovery rate correction**

The FDR controls the expected *proportion* of false positives among suprathreshold voxels.

$$FDR = E = \frac{\text{Falses positives}}{\text{Positives}} < q$$

A 0.05 FDR threshold ensures that, over many replications of the experiments, no more than 5% of the detected voxels will be false positives. The number of detections ("positives") will vary from experiment to experiment, and the exact percentage of false positives among the detections will vary, but on average, that percentage will not exceed 5%.

Determination of the correct threshold

First compute the contrasts of interest and look at the distribution of T values. Tom Nichols' scripts (FDRill.m & mulFDR.m) would help to determine the correct threshold. One can use the p-value calculated from the distribution of T scores in each voxel for one experimental condition (FDRill.m). One can also use a common threshold for different conditions using multFDR.m. This script computes a FDR threshold for a union of statistic images. Use multFDR cautiously: It says that you're willing to control false positives as a fraction of all detections in ***both*** images. This is potentially bad, as one image might have really strong, extensive signals, and the other image no signals, and then you'll have more false positives in one than the other.

## 6.3. Sphericity correction

What is sphericity and why to correct the sphericity?

The specified model (Y=B1X1 …+ e) postulates that the different composants are independent (or orthogonal). The decomposition of effects (main effects + interactions) is not valid if the different contrasts are correlated. If A is correlated with B, the model of independence Y=A+B is false because the real model is Y=A+C with C=(B - the part explained by A). Consequently, when using a model like Y=A+B, A would be intrinsically bigger than B. This difference between contrasts needs to be corrected.

How to correct it in SPM2?

The design matrix is pre-multiplied with a pre-whitening matrix. Performing a non-sphericity correction assumes non-identical variances and correlated repeated measures.
**Replications are over?**
**→ 'Pair' for paired t-test**
**→ 'Subjects' for ANOVA**

'Replications' are multiple occurrences of the same conditions. This is the dimension along which SPM2 does *not* need to estimate correlations, as observations in different subjects are independent. This means that you use your replications of your experiment, i.e. your subjects, to estimate the correlations between your repeated measures, i.e. the conditions.

**Correlated repeated measures**? → **'Yes'**
Here because the repeated measures are the within-subjects conditions, we need to take into account the correlation. There will therefore be non-zero off-diagonal elements in the variance-covariance matrix (i.e. correlations between the within-subject conditions). These are then estimated by SPM2 to enable pre-whitening.

## 6.4. Conjunctions

To perform a **conjunction** across subjects, press 'Results', and select several contrasts (pressing 'Control').

If 2 contrasts are selected SPM asks for:
**Null hyp. to assess?** Conjunction / Global

If 3 or more contrasts are selected SPM ask for:
**Null hyp. to assess?** Conj'n / Intermed / Global

If the **Global** button is used, a significant P-value indicates that one or more of the conjoined contrasts show an effect. SPM will compute corresponding thresholds for individual contrasts. For uncorrected thresholds, the individual threshold will be $p^{1/n}$, where p = individual threshold and n = number of contrasts in the conjunction. It must be assumed that the contrasts are independent or orthogonal; if they are not, use caution, as SPM will create a set of orthogonalized contrasts that may or may not be interpretable.

If the **Conjunction** button is used, a significant P-value indicates that *all* of the conjoined contrasts show an effect. Note that, by contrast with the global null hypothesis, contrasts do not need to be independent.

When the **Intermediate** button is used the user is asked to specify the number of "Effects under the Null", which is a number k, where 1<k<K, and K is the number of contrasts conjoined. If a significant P-value is found with the Intermediate button and a particular k, you can conclude that k or more of the contrasts show an effect. As with the Global button, you must assume that the contrasts are orthogonal.

# VII. Results and Graphs

In the Graphics window, SPM displays a **maximum intensity projection** (MIP) on a glass brain. The MIP is surfable: R-clicking in the MIP will activate a pulldown menu, L-clicking on the red cursor will allow it to be dragged to a new position. The design matrix (showing the selected contrast) is also surfable: R-clicking will show parameter names, L-clicking will show design matrix values for each scan.

In the SPM Interactive window a button box appears with various options for displaying statistical results (*p*-values panel), extract the time course of a given region (regional panel) and creating plots/overlays (visualisation panel).


## 7.1. Examine results

### *p*-values panel

To obtain a summary of local maxima, press '**volume**'. A list all clusters above the chosen level of significance as well as separate (>8mm apart) maxima within a cluster is display.
- x, y, z (mm): coordinates in MNI space for each maximum
- voxel-level: the chance (*p*) of finding a voxel with this or a greater height (*T*- or *Z*-statistic), corrected / uncorrected for search volume in a completely null statistic image.
- cluster-level: the chance (*p*) of finding a cluster with this or a greater size ($k_e$), corrected / uncorrected for search volume in a completely null statistic image.
- set-level: the chance (*p*) of finding this or a greater number of clusters (c) in the search volume in a completely null statistic image.

The '**cluster**' button will show coordinates and voxel-level statistics for local maxima (>4mm apart) in the selected cluster.

The '**small volume correction**' operates in new statistical analysis for a particular cluster or volume. Note that only corrected *p*-values will change.

- To look at T or F value for the whole brain, write the following into Matlab, and select the appropriate spmT or spmF images. The first 3 columns are MNI coordinates. The fourth is the statistic.

```
P=spm_get(1,'*.img','Select statistic image');
 V=spm_vol(P);
 [x,y,z] = ndgrid(1:V.dim(1),1:V.dim(2),0);
 for i=1:V.dim(3),
   z   = z + i;
   tmp = spm_sample_vol(V,x,y,z,0);
   msk = find(tmp~=0 & finite(tmp));
   if ~isempty(msk),
     tmp = tmp(msk);
     xyz1=[x(msk)'; y(msk)'; z(msk)'; ones(1,length(msk))];
     xyzt=V.mat(1:3,:)*xyz1;
     for j=1:length(tmp),
       fprintf('%.4g %.4g %.4g\t%g\n',xyzt(1,j),xyzt(2,j),xyzt(3,j),tmp(j));
     end;
   end;
 end;
```

**regional panel**

To extract a time course for data in a region of interest, select V.O.I. (*V*olume *O*f *I*nterest) and select ('don't adjust'): SPM displays a graph of the first eigenvariate of the data in or centered around the chosen voxel, and lists the eigenvariate values (Y) in the Matlab window.

## 7.2 Display results

**visualisation panel**

One can examine the size of effects with the **'plot'** button.

**1) Contrast of parameter estimates**: it shows effect size and error bars (in %).

**2) Fitted and adjusted response**: Plots adjusted data and fitted response across session/subject. If 'scan or time' is selected, the plot will show adjusted BOLD data (in %) and the regressor(s).

**3) Event/epoch-related responses**: Plots adjusted data and fitted response across peri-stimulus time. You can select:

**fitted response:** simple plot of mean (i.e., averaged over session) regressor(s) across PST

**fitted response and PSTH** (*p*eri-*s*timulus *t*ime *h*istogram): plots mean regressor(s) and mean signal +/- SE for each time bin.

Results can be obtained writing in the MatLab command prompt:
[Y,y,beta,SE,PST,PSTH,COL] = spm_graph(SPM,VOL,xX,xCon,xSDM,hReg)
The PSTH are the values of the PSTH graph and the PST values are the times.
PST and PSTH values can be save in a text file with fileout=[PST' PSTH']; save fileout.txt fileout -ascii

**fitted response +/- standard error:** plots mean regressor(s) **+/-** SE

**fitted response and adjusted data:** plots regressor(s) and individual

For anatomical visualisation of a cluster, press '**overlays**' which will activate a pulldown menu. There are three options:

**1) slices**: overlay on three adjacent (2mm) transaxial slices in the neuroradiological convention (modifications of the position are available in the left inferior part .. co-ordinates panel

To remove cross-hairs from activation maps type the following into the matlab command window: spm_orthviews('Xhairs','off')

To add cross hairs again, the command is: spm_orthviews('Xhairs','on')

**2) sections**: overlay on three intersecting (sagittal, coronal, transaxial) slices.

**3) render**: overlay on a volume rendered brain, with options for using a smoothed brain, and old and new style rendering.

Another possibility to display results and show different blods is to use the display menu. It is then possible to overlay several data onto the same brain.